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Review Article

# Comparative Lipid Analysis of Colostrum and Mature Human Milk Using UHPLC-Q-TOF-MS

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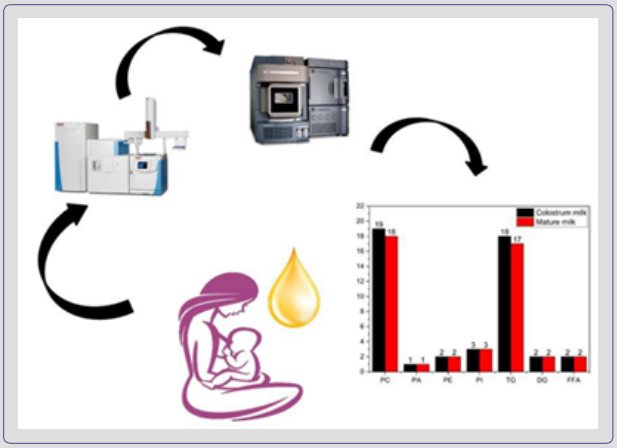
## Graphical Abstract




The evaluation of the lipid profile of colostrum and mature human milk was carried out using GC-FID and UHPLC-Q-TOF-MS techniques.

## Abstract

Human milk is a complete source of nutrients for newborns, considered an essential food for child development. One of the classes of nutrients present in milk are lipids, the main energy source for babies. This work presents a comparative study of the lipid profile of colostrum and mature human milk from Brazilian nursing mothers, using the techniques of gas chromatography with a flame ionization detector and ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. The major fatty acids in both milks were palmitic acid, oleic acid and linoleic acid. Furthermore, lipidomic analysis was carried out based on the fatty acid profile, where 48 different lipids were identified, classified as glycerophospholipids, glycolipids and non-esterified fatty acids. Comparing the lipid profile results, colostrum was more nutritious than mature milk in terms of lipid quality. The comparison of human milk through lipidomic analysis is still little explored in Brazil, however, it proved to be efficient in the present study, with the potential to further assist in understanding the processes of lipid absorption by the human body.

**Keywords:** lipid profile, human milk, ultra-performance liquid chromatography, gas chromatography with flame ionization detector

## Introduction

Human Milk (HM) is a complete source of nutrients for newborns, being considered a gold standard food during child growth. This food contains 3-5% (m/v) fat, mainly composed of Triacylglycerols (TAG), Diacylglycerols (DAG), Glycerophospholipids (GP), Sphingolipids (SP), nonesterified Fatty Acids (FFA), cholesterol and glycolipids [1]. TAGs are the main source of energy for babies and have in their structure different fatty acids located in the Sn-1, Sn-2 and Sn-3 positions, resulting in unique TAGs, with their digestion and absorption being influenced by these positions [2,3]. Because of this, Brazilian regulations from the National Health Surveillance Agency (ANVISA) determined that the practice of human milk donation be supported and encouraged in human milk banks. Mothers who cannot breastfeed due to infection with the Human Immunodeficiency Virus (HIV) or the human T-Cell Lymphotropic Virus (HTLV); use of some medications incompatible with breastfeeding; and/or insufficient milk production, they can make up for this lack of milk for babies with milk donated by milk banks. Donated milk can remain frozen for 15 days at a temperature of -18°C. It is worth mentioning that, due to the high perishability of HM, preservation methods, such as freeze-drying and spray-drying, have been developed with the aim of extending the shelf life of the food [4].

Several studies in different parts of the world have investigated the levels of fatty acids, total fat, phospholipids and cholesterol present in human milk. Additionally, some pooled data analyzes have been performed to evaluate lipids in human milk, but these analyzes have mainly focused on specific fatty acids such as EPA and DHA, total fat, or phospholipids. The composition of human milk is highly dynamic and changes according to diet, time of day and lactation period. Furthermore, this composition varies between individual mothers and between women of different ethnicities, being influenced by the

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mother's diet. Fat is one of the nutrients most subject to variations in human milk [5].

Lipids are present in human milk in the form of fat globules, which consist mainly of triglycerides surrounded by a structural membrane composed of phospholipids, cholesterol, proteins and glycoproteins. Human milk fat plays a significant role in infant nutrition, providing around 50 to 60% of energy intake, in addition to essential Fatty Acids (FAs) and fat-soluble vitamins. Triacylglycerols represent 98% to 99% of the total fat content in human milk and infant formulas and their properties depend on the length and degree of unsaturation of the fatty acids esterified in the glycerol skeleton, with long-chain fatty acids being Known as PUFAs, they are the most widely studied in human milk. Epidemiological studies have suggested that children exposed to higher concentrations of PUFAs in breast milk have better cognitive development [6,7], but other studies have indicated that extremely high concentrations of certain subtypes of PUFAs or of total PUFAs in colostrum have been associated with lower motor and cognitive performance [8], increased risk of developing allergic rhinitis and eczema [9] and other adverse outcomes such as sensitization, reduced lung function and increased fat mass [10]. Therefore, information on lipid profiles at different stages of lactation can provide guidance for establishing optimal nutrient intake for infants and serve as a basis for developing infant formulas.

Regarding the extraction of lipids from human milk using the Folch method, a mixture of chloroform and methanol is added to the milk sample and subjected to vigorous shaking to extract the lipids. The lipid phase is then separated from the aqueous phase by centrifugation and the solvent is removed by evaporation. Then, the resulting lipid residue is weighed and can be analyzed using gas chromatography and mass spectrometry. Folch extraction is an effective method for extracting lipids from human milk, both liquid and freeze-dried, as it is capable of extracting a wide variety of lipid classes, including fatty acids, phospholipids and cholesterol. Furthermore, the method is relatively simple, making it an attractive option for routine analysis in food analysis laboratories.

Combined with Folch extraction, for analyzing the lipid profile of human milk, new approaches in a more convenient and rapid way are essential. Therefore, lipidomics has been continuously used in numerous food approaches in order to investigate the lipid profile [11]. In this context, lipidomic analysis can provide important information about the quality of human milk, as it is the branch of science that studies the biochemical and molecular characterization of lipids in a given biological system, in addition to the lipid changes that are induced by several factors [12].

Some challenges regarding the complete and efficient extraction of the lipidome are considerable, such as differences in lipid structures and concentration levels. Therefore, careful sample preparation in lipidomics considerably helps to isolate the analytes of interest in order to simultaneously achieve high lipidome coverage and avoid signal suppression. Additionally, the sample preparation procedure for lipidomic analysis must be reproducible, robust and fast, enabling the extraction of a wide range of analytes with different polarities, molecular weights and different concentration levels [13]. Therefore, it is inferred that the objective of the study in question is to obtain and compare, through chemometric analyses, the lipid profile of lyophilized colostrum and mature human milk samples, with a view to carrying out lipidomic analysis using UPLC-MS/MS.

## Results and Discussion

### Fatty Acid Composition

A total of twenty-six fatty acids were identified and quantified by GC-FID in the colostrum and mature milk samples studied as elucidated in table 1.

| Fatty Acid | Colostrum | Mature |
|---|---|---|
| 04:00 | 0.10±0.01$^{b}$ | 0.14±0.03$^{a}$ |
| 06:00 | 0.34±0.01$^{b}$ | 0.61±0.08$^{a}$ |
| 10:00 | 0.37±0.01$^{b}$ | 0.56±0.00$^{a}$ |
| 12:00 | 2.88.0.08$^{b}$ | 3.49±0.06$^{a}$ |
| 14:00 | 4.81±0.09$^{a}$ | 4.85±0.10$^{a}$ |
| 14:1 | 0.20±0.00$^{a}$ | 0.20±0.00$^{a}$ |
| 15:00 | 0.14±0.01$^{b}$ | 0.23±0.01$^{a}$ |
| 16:00 | 25.04±0.41$^{a}$ | 22.65±0.45$^{a}$ |
| 16:1n-7 | 2.31±0.08$^{b}$ | 2.64±0.05$^{a}$ |
| 16:1n-9 | 0.17±0.02$^{b}$ | 0.59±0.03$^{a}$ |
| 17:00 | 0.46±0.01$^{a}$ | 0.30±0.04$^{b}$ |
| 18:00 | 6.13±0.16$^{b}$ | 8.95±0.13$^{a}$ |
| 18:1n-9 | 33.01±0.26$^{a}$ | 32.59±0.72$^{a}$ |
| 18:1n-7 | 1.99±0.02$^{a}$ | 2.22±0.30$^{a}$ |
| 18:2n-6 | 16.76±0.26$^{a}$ | 16.42±0.61$^{a}$ |
| 18:3n-3 | 0.71±0.01$^{a}$ | 0.43±0.06$^{b}$ |
| 18:3n-6 | 0.27±0.05$^{b}$ | 0.41±0.06$^{a}$ |
| 20:1n-9 | 1.52±0.09$^{a}$ | 0.99±0.78$^{b}$ |
| 20:3n-3 | 0.67±0.03$^{a}$ | 0.22±0.06$^{b}$ |
| 20:3n-6 | 0.36±0.02$^{a}$ | 0.36±0.00$^{a}$ |
| 20:5n-3 (EPA) | 0.11±0.00$^{a}$ | 0.09±0.00$^{b}$ |
| 22:00 | 0.82±0.04$^{a}$ | 0.42±0.01$^{b}$ |
| 20:3n-6 | 0.38±0.03$^{a}$ | 0.34±0.01$^{b}$ |
| 20:4n-6 (AA) | 0.08±0.01$^{a}$ | 0.09±0.00$^{a}$ |
| 22:5n-3 | 0.14±0.01$^{a}$ | 0.10±0.01$^{b}$ |
| 22:6n-3 (DHA) | 0.23±0.01$^{a}$ | 0.13±0.02$^{b}$ |
| ∑SFA | 41.09±0.91$^{a}$ | 42.20±0.27$^{a}$ |
| ∑MUFA | 39.20±1.88$^{a}$ | 39.23±0.73$^{a}$ |
| ∑PUFA | 19.71±0.84$^{a}$ | 18.59±0.57$^{a}$ |
| ∑n-3 | 1.89±0.15$^{a}$ | 0.97±0.19$^{b}$ |
| ∑n-6 | 17.85±0.68$^{a}$ | 17.62±0.36$^{a}$ |
| ∑n-6/∑n-3 | 9.60±1.02$^{b}$ | 18.16±0.44$^{a}$ |

**Table 1:** Fatty acid composition of colostrum and mature milk samples.

**Note:** Results expressed as mean ± standard deviation. Values with different letters in the same line show significant differences ($p<0.05$) using the Tukey test.

The colostrum and mature milk samples showed an SFA percentage of 41.09 and 42.20%, respectively. For MUFA the values were 39.20 and 39.23%, while for PUFA the results were 19.71 and 18.59%, respectively, as shown in table 1.

Caprylic acid (8:0) was not identified, and the presence of this FA in colostrum milk was not always identified, representing less than 0.1% of the total set of FAs, as reported in an analysis of combined data from 55 studies carried out globally [14]. Palmitic acid (16:0), among the SFAs, was the majority, presenting a concentration of 25.04 and 22.65% for colostrum and mature, respectively. Such

values are consistent with what was found in other related studies [4,15]. This fatty acid (16:0), when located in the Sn-2 position of TAG, helps with the absorption of calcium in the newborn's intestine, in addition to having an analgesic effect, as it increases the levels of the neurotransmitter anandamide, which has an analgesic effect [16].

As for MUFAs, oleic acid (18:1n-9) stood out, presenting concentrations of 33.01 and 32.59% for colostrum and mature, respectively. The values are close to those found by Manin et al. [4]. Furthermore, according to the study by Wu et al. [11], who investigated the lipid composition of donors from Taiwan, a content of approximately 27% oleic acid was observed. Oleic acid plays a crucial role in the development of the baby, being mainly responsible for providing energy to the newborn, helping to absorb fats from the intestine and acting as a structural component of the brain [4,17].

Regarding PUFAs, the main ones found in the present study were linoleic acids (18:2n-6) and α-linolenic acids (18:3n-3) with concentrations of 16.76 and 0.71% for colostrum milk and 16.42 and 0.43% for mature milk. These nutrients are considered essential, which means that they are not synthesized by the human body, that is, their intake is necessary through food. In addition, they play important roles in the child's immune system and hair growth and maintenance. Furthermore, these nutrients are precursors of Long-Chain Polyunsaturated Fatty Acids (LC-PUFA) such as arachidonic acid (AA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) [4,18].

Regarding the AA, DHA and EPA values found, the results for colostrum milk were 0.08; 0.11 and 0.23%, respectively. For mature milk, the values were 0.09; 0.09 and 0.13%. In a study conducted by Duan et al. [19], in South Korea, the compositions of fatty acids (FA) in human milk from lactating mothers in Korea were analyzed, resulting in Arachidonic Acid (AA), Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) levels of approximately 0.54%, 0.22% and 0.77%, respectively. Thus, as the diet of Korean mothers is different from the diet of Brazilian mothers, some acid values found in this study may differ from the literature, given that the diet of lactating mothers directly interferes with the lipid profile of human milk.

It is important to highlight that there was no significant difference ($p<0.05$) in the levels of SFA, MUFA and PUFA in both milks analyzed. However, in the case of the major fatty acids, there was a significant decrease in the content of palmitic acid (16:0) in colostrum milk for mature milk, but oleic acids (18:1n-9) and linoleic acids (18:2n-6) showed no significant change. Regarding the content of omega-3 fatty acids, there was a significant decrease, from 1.89% to 0.97%. Thus, for the newborn, colostrum milk, in addition to all the benefits already mentioned in the literature, is more nutritious in terms of lipid quality.

## Lipid Composition by LC-MS/MS

Through an exploratory analysis, 48 different lipids were identified, as detailed in table S1 (see supplementary material). Classified into three lipid categories (GL, GP and FAA), there were two subclasses of GLs (Diacylglycerol [DG] and Triacylglycerol [TG]) four subclasses of GP (Phosphatidic acid [PA], Choline Glycerophospholipid [PC], Ethanolamine Glycerophospholipid [PE], Phosphatidylinositol [PI], and the Fatty Acids Free-range (FFA); including 19 PC species (18 for mature milk), two FFA species, two PE species, one PA species, three PI species, two DG species and 18 TG species (17 for mature milk). Figure 1 illustrates the quantity of compounds found for each lipid class.

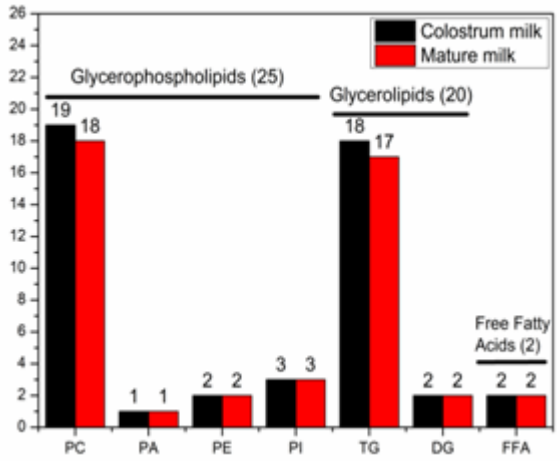


**Figure 1:** Lipid subclasses identified in colostrum and mature milk.

Around 95 to 98% of the fat present in human milk are TGs. Among the TGs identified, those that contain palmitic acid (16:0) in the Sn-2 position stand out, given the importance of this TG in this position to aid calcium absorption in the intestine of newborns. Assistance in the process of calcium absorption in the intestine of neonates, along with the ability to induce an analgesic effect, is possible due to increased levels of anandamide, a neurotransmitter with analgesic properties.

TGs in human milk have a unique structure associated with efficient absorption and utilization of FAs. In the present study, TGs in human milk were identified by UPLC-Q-TOF-MS.

As shown in figure 1, through exploratory analysis, the most abundant TGs in colostrum milk were L-P-L (18:2/16:0/18:2) followed by O-L-L (18:1/18:2/18:2) represented by one of its isomeric forms with 16.93% and 9.76% of the total TGs respectively. For mature milk, the TGs highlighted were TG O-32:8 with 18:81% and L-P-L (18:2/16:0/18:2) with 15.82% of the total TAGs.

It is worth highlighting the presence of palmitic acid, one of the main TGs found in both human milk samples, in the Sn-2 position, mainly influencing endogenous hormonal functions. Therefore, it is inferred that the neonate may have good digestibility, as palmitic acid in the Sn-2 position is more easily digested by the body compared to other fatty acids in the Sn-1 or Sn-3 position. The presence of palmitic acid in the Sn-2 position helps to increase the efficiency of digestion and absorption of TGs. Our findings similarly agree with the study by Castro et al. [20], through research on breast milk.

Furthermore, there is also the presence of linoleic acid in the same position, and there are many benefits when a PUFA is located in the Sn-2 position, given that there is a low activity of pancreatic lipase in relation to these fatty acids when present in the Sn-1 and Sn-3 positions. Thus, due to the action of lipases, the fatty acid in the Sn-2 position has greater bioavailability, being more easily absorbed by the newborn's body.

Furthermore, some compounds may present different interactions in different samples. Therefore, to highlight the identification of the lipid profile of colostrum and mature human milk samples, analyzing

patterns and relationships between samples and variables, heat map analysis was performed. Figure 2 below shows a heat map of the 48 lipids found in human milk. Heat maps are useful tools in chemical analysis and interpretation of multivariate data. They are two-dimensional graphs that present color information to represent the magnitude of a given value in a data array. In analytical chemistry, heat maps are often used to visualize patterns in spectral data, such as mass spectra obtained in the present analysis, helping to compare data from different samples, experiments or treatments in an analysis [21].

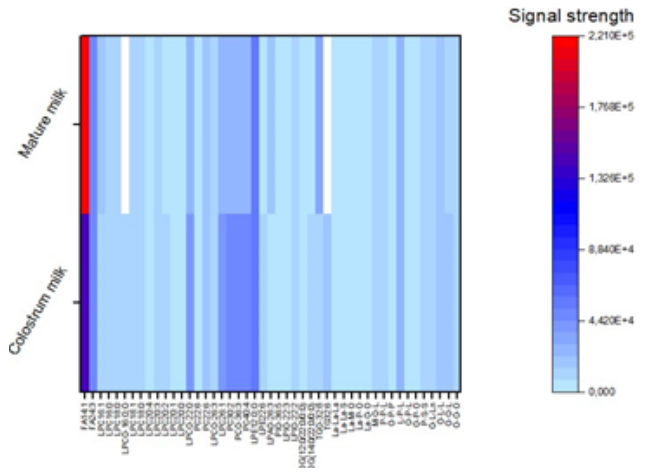


**Figure 2:** Screening results (heat map) of lipids present in human milk from the colostrum and mature lactation phases.

**Note:** Bu, 4:0; Co, 6:0; Cy, 8:0; Ca, 10:0; La, 12:0; M, 14:0; P, 16:0; Po, 16:1; O, 18:1; S, 18:0; L, 18:2; Ln, 18:3; Eo, 20:1; Ed, 20:2; Et, 20:3; ARA, 20:4; EPA, 20:5; DPA, 22:5; DHA, 22:6.

The results in figure 2 reveal that human milk in the different lactation phases analyzed differ in terms of lipid composition. However, they also demonstrate some similarities to lipid levels when looking at individual components. The columns in the heat map represented, through colors, the amounts of each lipid component in the samples, allowing us to infer which of them each constituent was present in greater abundance, being represented by darker tones.

A main difference is in the 14:1 fatty acid, which was found to be higher in mature milk. However, TG 32:6 and LPC O-16:0;O were found only in colostrum milk, showing an obvious difference in the heat map. Other compounds showed significant differences between milk samples, such as PC 40:4, PC O-36:3, PC 30:2, LPC 26:1 and LPC O-22:0, which were present in greater quantities in milk colostrum. From the glycerolipid class, the same TGs found in the present study were also found in the research by Yuan et al. [22].

Regarding the class of glycerophospholipids, in the research carried out by Song et al. [23], a phospholipidomic analysis was carried out on breast milk collected from Chinese mothers, finding 258 different phospholipids, and the 25 compounds of this class found in the present research are consistent with the mentioned work, with the exception of PC30:2 (12:0/18 :2) and LPE12:0. This may be linked to the fact that the amount of AG 18:2n-6 is present in large quantities in the analyzed milk, leading to the presence of this AG in the phospholipid structure. It is worth mentioning that depending on the region and location, mothers' diets can be completely different from each other, causing changes in the lipid profile of breast milk. Lipidomic analysis to differentiate the phases of breast milk has been little explored, with no reports found in the literature of such analysis in Brazil, for example. Therefore, the present research can be a pioneer in this sense.

## Experimental

### Materials, Solvents and Reagents

Methanol, chloroform, anhydrous sodium sulfate, and sodium hydroxide were purchased from Sigma-Aldrich (Darmstadt, Germany). Standard analytical mixture of Fatty Acid Methyl Esters (FAMES 189-19) was purchased from Millipore-Sigma (St. Louis, USA). For chromatographic analysis, all reagents and solvents used were of analytical grade (purity ≥99.9 %), while mass spectrometry analysis was performed using HPLC grade solvents.

The research ethics committee of the State University of Maringá approved all procedures with process number 3,430,478. Initially, in the human milk bank of the Hospital Universitário de Maringá (Maringá, Paraná, Brazil) samples of human milk from the lactation phase, colostrum and mature human milk were collected under cooling at 4ºC according to a specific protocol for the human milk bank under regulations from ANVISA [24], totaling a total volume per phase of 1200 mL. Subsequently, a homogenized pool was formed and divided into appropriate containers for the proposed processing of colostrum and mature human milk.

A pool volume of colostrum and mature milk were subjected to the freeze-drying process according to Manin et al. [25], at approximately -54°C and 0.021 mbar for 48 h in the *Enterprise I* freeze-dryer. continuous until constant weight. The powdered milk was vacuum packed in light-free aluminum bags, frozen at -18°C, for later analysis. The dried samples were subjected to Folch extraction, as well as subjected to subsequent lipidomic analysis.

### Extraction of Lipids

The method of Folch et al. [26], he was adopted for the extraction of lipids from milk samples, using a sample:solvent ratio of 1:5 (v/v) and the use of chloroform:methanol extraction solvents in the 2:1 (v/v) ratio. For each sample, 10g of freeze-dried milk was mixed with 50 mL of the chloroform:methanol solution and stirred for 15 minutes. Then, the mixture was centrifuged for 5 minutes at 6000rpm and a temperature of 25°C. Subsequently, 10mL of 1% $Na_2SO_4$ solution was added to the solution containing the sample, shaking again for 5 minutes and centrifuging under the same conditions. After phase separation, the lower phase was collected in a previously weighed 250mL flask, and the extracting solvent mixture was evaporated at an appropriate temperature, using a rotary evaporator. The lipid fractions were collected using a pipette and a small volume of hexane, transferred to an Eppendorf and stored at -18°C in a freezer until analysis was carried out.

### Lipid Derivatization

The proposed methodology for derivatization of fatty acids present in the milk sample consists of lipid methylation, using the method described in ISO 5509 (1978). To obtain fatty acid methyl esters, 100mg of lipids extracted in test tubes were added, along with 2mL of n-heptane and 500µL of methyl tricosanoate (23:0; ≥99% Sigma-Aldrich, Darmstadt, Germany) as internal standard. The test tubes were shaken for 2 minutes in a VX-200 vortex (Labnet International Inc, New Jersey, USA). Then, 2mL of esterifying reagent (KOH/MeOH 2mol $L^{-1}$) was added to the solution, which he stirred again under the same

conditions and period. After the derivatization process, the upper (organic) phase was collected for GC-FID analysis.

### Composition in Fatty Acids

The analysis of the fatty acid composition was carried out through chromatographic separation and identification using a Shimadzu GC-2010 Plus gas chromatograph equipped with a flame ionization detector and fused silica capillary column (Select FAME, 100 m x 0.25 mm i.d x 0.25µm cyanopropyl film thickness) and split/splitless injection system. A split ratio of 1:100 and injection volume of 2µL were used. The injector and detector temperatures were set at 240ºC and 250ºC, respectively. The carrier, auxiliary, synthetic air and hydrogen gasses were used with flows of 1.4mL. $min^{-1}$, 30mL. min-$^{1}$, 300mL. $min^{-1}$ and 30mL. $min^{-1}$, respectively. Chromatographic separation was performed with the following heating schedule: the column was heated to 65ºC and held stable for 4min, then a heating ramp of 16ºC $min^{-1}$ was used to raise the column temperature to 185ºC. After 12min, a new heating ramp of 20ºC $min^{-1}$ was used so that the column temperature reached 235ºC. This temperature was maintained for 9min, totaling an analysis time of 35min. Analytical standard methyl tricosanoate (23:0; ≥99% Sigma-Aldrich, Darmstadt, Germany) was used as an internal standard for quantification. To interpret the results obtained by GC-FID, the Chromquest 5.0 software was used and the results analysis were expressed as a relative percentage of total fatty acids.

### Lipidomic Analysis

100mg of the samples were resuspended in 10ml of isopropanol and 10µl of each extract was injected and analyzed using ultra-high performance liquid chromatography (Shimadzu, Nexera X2, Japan). Separation of individual components was performed using an Acquity UPLC® BEH C18 (Waters, EUA, 1.7µm, 2,1 × 50mm) at a flow rate of 0.300mL $min^{-1}$. The analysis was carried out in isocratic mode with 1% of solvent A and 99% of solvent B, being 0.1% ammonium formate in acetonitrile (solvent A) and 0.1% ammonium formate in isopropanol (solvent B), with the column temperature maintained at 55°C.

MS experiments were performed on an Impact II high-resolution mass spectrometer of Q-TOF geometry (Bruker Daltonics Corporation, Germany) equipped with an electrospray ionization source. The instrument was calibrated using a sodium formate solution (10mmol $L^{-1}$ NaOH solution in 1:1 (v/v) isopropanol:water solution containing concentrated formic acid). The source was operated in positive ionization mode. Drying gas parameters were set to 450 L $h^{-1}$ at 250°C and mist gas pressure at 4 bar. The source temperature was maintained at 130°C, capillary voltage at 3.00kV and cone voltage at 35.0 V. Data were collected in the range of *m/z* 100 to 1000 with an acquisition rate of 5Hz and the most intense ions were used for Multiple Reaction Monitoring (MRM), using a staggered program with collision energies ranging from 15 to 40 eV. The data obtained through an exploratory analysis were attributed using the LAMES Platform, based on the mathematical algorithm that elucidates the distribution of fatty acids in TAG molecules. Furthermore, the LipidMaps® database [27], was used, which made it possible to find the molecular formula of TAGs in colostrum and mature human milk samples for lipidomic analysis.

### Statistical Analyzes

In this study, triplicate analyzes were carried out, with the aim of guaranteeing the precision and reliability of the results obtained. The results obtained from the fatty acid composition were presented as mean ± standard deviation. To assess the significance of the results, we applied Analysis of Variance (ANOVA), followed by the Tukey test to compare means.

Regarding multivariate analyses. The Origin software (OriginLab Corporation), was used to design the graphs and heatmap, in order to identify differences between the analyzed samples, differences in the extraction method and identification of possible patterns in the samples.

## Conclusion

The results of this study obtained through Multiple Reaction Monitoring (MRM) through UPLC-Q-TOF-MS equipped with an electrospray ionization source combined with lipidomic analysis demonstrated distinctions between the lipid profile for the sample groups of human milk colostrum and mature human milk. In general, colostrum milk had the highest proportion of phospholipids, as well as the highest content of omega-3 fatty acids, obtaining superior nutritional quality when compared to mature milk. Given the need of the newborn in the first days of life, our findings are necessary through the nutritional investigation of milk from Brazilian nursing mothers. Furthermore, although lipidomic analysis has already been addressed in breast milk around the world, in Brazil it is little explored, thus considering the present research as one of the initial researches in this area in the country to provide a deep understanding of the lipid composition of breast milk. human milk from the colostrum and mature phases, and its dynamic changes based on lipidomics.

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## Author's Contribution

Geovane A. R. da Silva was responsible for the conceptualization, data curation, investigation, methodology, formal analysis, investigation, writing - review editing, project administration; Patricia D. S Santos was responsible for the formal analysis and writing; Amanda C. Assakawa for the writing - review & editing; Alisson L. Figueiredo for the writing - review & editing; Cintia Stefhany Ripke Ferreira: Conceptualization. Writing – original draft; Jesui V. Visentainer for the resources and funding acquisition; Oscar O. Santos was responsible for the conceptualization, writing - review & editing, supervision and funding acquisition.

## Declarations

### Conflict of Interest

No conflict of interest.

### Ethical Approval

Not applicable.

## References

1. Wang L, Li X, Liu L, Zhang H, Zhang Y, et al. (2020) Comparative lipidomics analysis of human, bovine and caprine milk by UHPLC-Q-TOF-MS. Food Chem 310: 125865.

2. Vyssotski M, Bloor SJ, Lagutin K, Wong H, Williams DBG (2015) Efficient separation and analysis of triacylglycerols: Quantitation of β-palmitate (OPO) in oils and infant formulas. J Agric Food Chem 63: 5985-5992.

3. Zhao P, Zhang S, Liu L, Pang X, Yang Y, et al. (2018) Differences in the triacylglycerol and fatty acid compositions of human colostrum and mature milk. J Agric Food Chem 66: 4571-4579.

4. Manin LP, Rydlewski AA, Pizzo JS, Cruz VHM, Alves ES, et al. (2023) Effects of pasteurization and high-pressure processing on the fatty acids, triacylglycerol profile, Dornic acidity, and macronutrients in mature human milk. J Food Compos Anal 115: 104918.

5. Zhang Z, Wang Y, Yang X, Cheng Y, Zhang H, et al. (2022) Human milk lipid profiles around the world: A systematic review and meta-analysis. Adv Nutr 13: 2519-2536.

6. Zielinska MA, Hamulka J, Grabowicz I, Brys J, Wesolowska A (2019) Association between breastmilk LC PUFA, carotenoids and psychomotor development of exclusively breastfed infants. Int J Environ Health Res 16: 1144.

7. Guxens M, Mendez MA, Moltó-Puigmartí C, Julvez J, García-Esteban R, et al. (2011) Breastfeeding, long-chain polyunsaturated fatty acids in colostrum, and infant mental development. Pediatrics 128: 880-889.

8. Bernard JY, Armand M, Garcia C, Forhan A, Agostini MD, et al. (2015) The association between linoleic acid levels in colostrum and child cognition at 2 and 3 y in the EDEN cohort. Pediatr Res 77: 829-835.

9. Waidyatillake NT, Stone YR, Thien F, Lodge CJ, Simpson JA, et al. (2017) Breast milk polyunsaturated fatty acids: Associations with adolescent allergic disease and lung function. Allergy 72: 1193-1201.

10. Much D, Brunner S, Vollhardt C, Schmid D, Sedlmeier E, et al. (2013) Breast milk fatty acid profile in relation to infant growth and body composition: Results from the INFAT study. Pediat Res 74: 230-237.

11. Wu D, Zhang L, Tan CP, Zheng Z, Liu Y (2023) Comparative lipidomic analysis reveals the lactational changes in the lipid profiles of chinese human milk. J Agric Food Chem 71: 5403-5416.

12. Wenk MR (2010) Lipidomics: New tools and applications. Cell 143: 888-895.

13. Bakhytkyzy I, Hewelt-Belka W, Kot-Wasik A (2020) The dispersive micro-solid phase extraction method for MS-based lipidomics of human breast milk. Microchem J 152: 104269.

14. Floris LM, Stahl B, Abrahamse-Berkeveld M, Teller IC (2020) Human milk fatty acid profile across lactational stages after term and preterm delivery: A pooled data analysis. Postaglandins, Leukot Essent Fat Acids 156: 102023.

15. Demmelmair H, Koletzko B (2018) Lipids in human milk. Best Pract Res Clin Endocrinol Metab 32: 57-68.

16. Visentainer JV, Santos OO, Maldaner L, Zappielo C, Neia V, et al. (2018) Lipids and fatty acids in human milk: Benefits and analysis. IntechOpen, London, UK.

17. Jensen RG (1999) Lipids in human milk. Lipids 34: 1243-1271.

18. Silva RC, Escobedo JP, Gioielli LA, Quintal VS, Ibidi SM, et al. (2007) Centesimal composition of human milk and physico-chemical properties of its fat. Quim Nova 30: 1535-1538.

19. Duan B, Shin J, Qin Y, Kwon J, Lee K (2019) A study on the relationship of fat content in human milk on carotenoids content and fatty acid compositions in Korea. Nutrients 11: 2072.

20. Castro MC, Oliveira FS, Alves ES, Zacarias JMV, Alencar JS, et al. (2023) Influence of breastfeeding time on caloric composition and IL-10 and TNF-α cytokines, fatty acids, and triacylglycerol in human milk colostrum in previous, intermediate, and posterior milk. J Braz Chem Soc 34: 201-212.

21. Kumar N, Bansal A, Sarma GS, Rawal RK (2014) Chemometrics tools used in analytical chemistry: An overview. Talanta 123: 186-199.

22. Yuan Y, Xu F, Jin M, Wang X, Hu X, et al. (2021) Untargeted lipidomics reveals metabolic responses to different dietary N-3 PUFA in juvenile swimming crab (Portunus Trituberculatus). Food Chem 354: 129570.

23. Song S, Liu T, Liang X, Liu Z, Yishake D, et al. (2021) Profiling of phospholipid molecular species in human breast milk of Chinese mothers and comprehensive analysis of phospholipidomic characteristics at different lactation stages. Food Chem 348: 129091.

24. Anvisa (2006) National Health Surveillance Agency. Resolution of the collegiate board - RDC N° 171.

25. Manin LP, Rydlewski AA, Galuch MB, Pizzo JS, Zappielo CD, et al. (2019) Evaluation of the lipid quality of lyophilized pasteurized human milk for six months by GC-FID and ESI-MS. J Braz Chem Soc 30: 1579-1586.

26. Folch J, Less M, Sloanestanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226: 497-509.

27. Lipidmaps Database (2024) A free, open access lipidomics resource.

## Supplementary Information

| No. | Molecular Species | Mode | m/z in MS/MS (Precusure Ion>Product Ion) | MS Signal Strength | |
|---|---|---|---|---|---|
| | | | | Colostrum | Mature |
| 1 | FA14:1 | $[M+H]^+$ | 226.9517>90.9766 | 145224 | 220133 |
| 2 | FA24:3 | $[M+H]^+$ | 362.9267>158.9641 | 44704 | 44704 |
| 3 | LPC16:1 | $[M+H]^+$ | 493.4314>184.1000 | 12456 | 15436 |
| 4 | LPC16:0 | $[M+H]^+$ | 495.3808>184.1000 | 13458 | 12456 |
| 5 | LPC18:0 | $[M+H]^+$ | 507.4370>184.1000 | 14258 | 14146 |
| 6 | LPCO-16:0;O | $[M+NH4]^+$ | 515.4896>143.0162 | 12450 | nd |
| 7 | LPC18:1 | $[M+H]^+$ | 521.4271>184.1000 | 8759 | 12320 |
| 8 | LPC18:0 | $[M+H]^+$ | 523.6031>184.1000 | 9635 | 8754 |
| 9 | LPC20:4 | $[M+H]^+$ | 531.5106>184.1000 | 4988 | 3489 |
| 10 | LPC20:3 | $[M+H]^+$ | 535.4487>184.1000 | 10256 | 11132 |
| 11 | LPC20:2 | $[M+H]^+$ | 547.4786>184.1000 | 7598 | 5899 |
| 12 | LPC20:1 | $[M+H]^+$ | 549.4604>184.1000 | 3512 | 2835 |
| 13 | LPC20:0 | $[M+H]^+$ | 551.9000>184.1000 | 2589 | 2550 |
| 14 | LPCO-22:0 | $[M+H]^+$ | 566.8891>226.9515 | 39818 | 27109 |
| 15 | PC22:6 | $[M+H]^+$ | 582.4052>337.4000 | 2100 | 1589 |
| 16 | PC22:6 | $[M+H]^+$ | 609.5527>337.5000 | 18756 | 19321 |
| 17 | LPCO-26:3 | $[M+NH4]^+$ | 633.5053>338.4200 | 11364 | 12223 |
| 19 | LPC26:1 | $[M+H]^+$ | 634.8767>226.9515 | 38296 | 27366 |
| 20 | PC30:2 | $[M+H]^+$ | 702.8642>226.9515 | 51398 | 27670 |
| 21 | PCO-36:3 | $[M+H]^+$ | 770.8516>226.9515 | 45396 | 28842 |
| 22 | PC40:4 | $[M+H]^+$ | 838.8392>226.9515 | 44310 | 28986 |
| 23 | LPE12:0;O | $[M+NH4]^+$ | 430.9141>90.9766 | 56035 | 55055 |
| 24 | LPE22:6 | $[M+H]^+$ | 526.8000>184.1000 | 7456 | 6354 |
| 25 | LPAO-26:3 | $[M+H]^+$ | 531.4059>143.0159 | 3158 | 17045 |
| 26 | PIO-36:5 | $[M+H]^+$ | 635.8790>337.4600 | 5478 | 7124 |
| 27 | LPIO-22:3 | $[M+H]^+$ | 637.8814>339.4800 | 2497 | 2153 |
| 28 | LPIO-22:2 | $[M+H]^+$ | 639.6080>339.4800 | 12546 | 11421 |
| 29 | DG(12:0/22:0/0:0) | $[M+NH4]^+$ | 613.5886>340.4200 | 2155 | 1543 |
| 30 | DG(14:0/22:0/0:0) | $[M+NH4]^+$ | 641.1476>341.4800 | 7453 | 6590 |
| 31 | TGO-32:8 | $[M+H]^+$ | 553.3894>313.1800 | 9572 | 32087 |
| 32 | TG32:6 | $[M+NH4]^+$ | 588.3546>226.9515 | 16460 | nd |
| 33 | La-La-La | $[M+NH4]^+$ | 657.4655>439.6500 | 1255 | 1010 |
| 34 | La-La-S | $[M+NH4]^+$ | 739.9958>439.5400 | 3568 | 3975 |
| 35 | La-M-O | $[M+NH4]^+$ | 767.8434>467.6800 | 2578 | 2122 |
| 36 | La-P-O | $[M+NH4]^+$ | 795.5129>495.6600 | 3365 | 3318 |
| 37 | La-O-O | $[M+NH4]^+$ | 821.9964>521.7200 | 3588 | 2656 |
| 38 | M-O-L | $[M+NH4]^+$ | 847.1134>549.7600 | 11245 | 10374 |
| 39 | P-P-L | $[M+NH4]^+$ | 849.9689>549.7600 | 9989 | 10326 |
| 40 | O-P-P | $[M+NH4]^+$ | 851.7112>551.8400 | 2654 | 2261 |
| 41 | L-P-L | $[M+NH4]^+$ | 873.3343>575.7700 | 28556 | 26986 |
| 42 | O-P-L | $[M+NH4]^+$ | 875.9722>575.7700 | 4123 | 3560 |
| 43 | O-P-O | $[M+NH4]^+$ | 877.4678>577.7300 | 4586 | 4678 |
| 44 | P-S-S | $[M+NH4]^+$ | 880.1645>577.7300 | 11088 | 11067 |
| 45 | O-L-Ln | $[M+NH4]^+$ | 896.6918>597.7500 | 10265 | 10245 |
| 46 | O-L-L | $[M+NH4]^+$ | 899.5013>339.3500 | 16458 | 17536 |
| 47 | O-O-L | $[M+NH4]^+$ | 901.8028>766.4800 | 15360 | 14596 |
| 48 | O-O-O | $[M+NH4]^+$ | 903.8215>603.7700 | 13889 | 13748 |

**Table S1:** Lipid species identified in colostrum and mature milk[a].

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